

BEHAVIORAL SPECIFICITY AND THE INDUCTION OF HOST RECOGNITION IN A SYMBIOTIC POLYCHAETE¹

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Chemically mediated behavioral phenomena are common throughout the animal kingdom and have been the subjects of numerous recent reviews (Davenport, 1966; Butler, 1967; Blun, 1969; Lenhoff, 1968; Schoonhoven, 1968; Regnier and Law, 1968; Gleason and Reynierse, 1969). These investigations have clearly demonstrated the importance of specific chemical information in the mediation of countless inter- and intraspecific relationships. Indeed, chemical communication appears to be the paramount mode of communication in most groups of animals (Wilson, 1970).

Several kinds of chemically mediated phenomena are known to be susceptible to modification through some kind of conditioning process. Thorpe and Jones (1937) introduced the concept of "olfactory conditioning" to describe the effect of exposure to an abnormal host on the subsequent host selection behavior of an insect parasite. Similarly, Cushing (1941) invoked such a mechanism to explain substrate preference for oviposition by *Drosophila*. Numerous additional accounts of the role of previous experience in various chemically mediated behavioral phenomena appear in the literature on insect behavior (Dethier, 1970).

Selection of the particular stream leading to their birthplace by anadromous fishes (Hasler and Wisby, 1951), food preference in various vertebrates (Ivlev, 1961; Burghardt, 1966; Burghardt and Hess, 1966), and prey selection by three predatory marine invertebrates, the Pacific starfish *Pisaster* (Landenberger, 1968) and the gastropods *Urosalpinx* (Wood, 1968) and *Acanthina* (Murdoch, 1969), are additional chemically mediated phenomena affected by conditioning. The concepts "chemical imprinting" (Burghardt, 1966) and "ingestive conditioning" (Wood, 1968) have been introduced into the literature as a result of these kinds of investigations. It appears that these chemical conditioning phenomena are widespread throughout the animal phyla.

It has been clearly demonstrated (see Davenport, 1966) that several groups of symbiotic polychaetes are capable of recognizing and responding to some chemical signal emanating from their hosts. Among these polychaetes the polynoid genus *Arctonoe* presents an interesting complex of host-symbiont associations that are amenable to comparative experimental analyses. One species in this genus, *Arctonoe pulchra*, is of particular interest in that this worm is associated with at least nine species representing five classes in three phyla (Pettibone, 1953; Dimock, 1970). Previous work with this species has been limited to a demonstration of a

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chemotactic response by *A. pulchra* to one of its hosts, the sea cucumber *Stichopus californicus* (Davenport, 1950; Davenport and Hickok, 1951). In the investigations reported here populations of this worm from four additional host species have been examined with regard to the occurrence of chemically mediated host recognition behavior, the specificity of these responses, and the effect of the past experience of individual worms on their subsequent host recognition behavior.

METHODS

Several designs of olfactometers were tested for use as an assay for the host recognition behavior of *Arctonoe pulchra*. It soon became obvious that any apparatus employing multiple worms simultaneously was of no use with this species as these worms are particularly aggressive towards each other and cannot be confined with one another. The design finally settled upon was a modification of the Y-maze choice apparatus described by Davenport (1950). This device, constructed of $\frac{1}{2}$ inch i.d. Lucite tubing, consisted of a 10 cm stem connected to two 8 cm arms of the Y 60° apart. The arms terminated at a 45° elbow. The stem was fitted with a partial partition and drain post at its free end. The whole device was mounted on a sheet of $\frac{1}{4}$ " Lucite provided with 3 brass bolt legs for leveling. Thus, test solutions could be introduced at the arms of the Y, the device leveled, and the solutions allowed to drain slowly from the stem, a procedure which resulted in the apparatus being maintained with a slowly flowing solution which filled the tube to approximately $\frac{1}{3}$ to $\frac{1}{2}$ full.

There are several inherent limitations and difficulties associated with a choice apparatus of this design. Since test organisms must be employed one at a time in such a device, considerable time may be spent in amassing enough data for statistical analysis. Depending upon the arrangement of the organism's chemoreceptors which mediate the response under investigation, it is possible that the subject could be stimulated by effluents from only one of the two arms of the maze. It is also quite possible that an individual organism may exhibit a preference for either the left or the right arm of the Y-maze (Putnam, 1962), a preference that may either be genetic or simply a response to some trails or traces from animals in previous trials. Occasionally, an animal may make an over-shoot mistake at the junction of the two arms as a result of the sudden confrontation at the sharp demarcation between two alternate currents. The most important limitation, however, is the lack of an opportunity for the analysis of the full behavior of the animal evoked by chemical signals, since the response is limited to a directed response canalized to a simple "choice" (Gage, 1966).

These limitations have been acknowledged in this study and efforts made to minimize their effect on the interpretation of the results. Individual worms have not been used repeatedly in single experiments. The presentation of test solutions into the arms of the Y-maze was randomized. The tubes were thoroughly rinsed in clean sea water between individual tests. Finally, the observed responses are recognized as being only part of a behavior pattern which might effect contact between a symbiont and its host and are not considered to be the sole basis of host recognition by these organisms.

Test solutions (hereafter referred to as test or host effluents) were prepared by allowing test organisms (enough total mass to displace 500 ml) to stand in 3000 ml

of aerated filtered sea water for 20–24 hours prior to testing. Any irregularity in the production of an attractant by a test organism was compensated for by using this lengthy time period and typically 2 or 3 test organisms per 3000 ml. Effluent thus prepared was then siphoned into the arms of the Y-maze. Since preliminary experiments suggested that there was little effect of flow rate on the worms' behavior, the flows in the two arms of the Y were arbitrarily balanced at 0.5 ml/arm/7–10 sec with polyethylene buret tips. Temperatures were regulated by water baths (11–14° C, Friday Harbor; 15–19° C, Santa Barbara).

The organisms employed in these experiments were collected by hand while diving or were dredged. In Santa Barbara, California, the sea cucumber *Stichopus parvimensis* and the limpet *Megathura crenulata* with their symbionts were collected from Naples Reef, a subtidal reef lying approximately 20 km west of Santa Barbara. The sea star *Dermasterias imbricata* was collected from under the Signal Oil company pier at Ellwood, California. At Friday Harbor, Washington, the sea star *Petalaster* (= *Luidia*) *foliolata* was dredged from Bellingham Bay and the sea cucumber *Stichopus californicus* was dredged from East Sound, Orcas Island. Additional *Stichopus* and all other organisms employed in experiments at Friday Harbor were collected by diving in the vicinity of San Juan Island.

All experimental organisms were maintained in running sea water aquaria. Test organisms were replaced about every 2–3 weeks and no excessive mortality of either worms or their hosts occurred during this time. No worms were used more than once in a particular experiment; however, several experiments utilized the same worms. Worms were removed from their hosts and placed in individual dishes of filtered sea water for at least one hour prior to testing. The water in the dishes was replaced at least once during this period to remove any traces of host secretions. This was found to be necessary particularly with the worms from the mollusc host, since these worms frequently were covered with host mucus. The worms were then introduced into the Y-maze (with test solutions flowing) and their distribution recorded after a ten minute experimental period. A worm which had not entered either arm of the Y (enter = an arbitrary $\frac{1}{3}$ worm's length in the arm) was scored as a negative.

Providing no bias exists in the choice apparatus and each trial is independent of any other trial, the distribution of the worms in the two arms of the maze will approximate a binomial distribution with $P = q = 0.5$. The probability of getting the observed distributions of the worms in particular experiments has thus been evaluated by a chi-square goodness-of-fit analysis. The χ^2 value is the sum of the χ^2 values for the distribution of worms in each arm of the maze, assuming an expected value of 50% of the total number of worms choosing. The probability associated with this χ^2 value is then found by entering the table of χ^2 at 1 degree of freedom.

RESULTS

Chemotactic responses to test effluents

Host recognition. Host recognition behavior of *Arctonoe* was monitored by analyzing the responses of the experimental populations of this symbiont to test effluent in the choice apparatus. In this series of experiments worms were simul-

taneously exposed to filtered sea water and to effluent from their respective hosts. These results, tabulated in Table I, clearly indicate that except for the population of *Arctonoe* living with the sea star *Petalaster*, all of the experimental populations of this symbiont exhibited a significant preference for the arm of the Y containing water in which their original host was being maintained. The worms from *Petalaster* not only failed to show any overt recognition of the effluent from their host but also exhibited no response which could be interpreted as recognition when offered tubefeet excised from this star or sponge swabs bathed in host mucous.

Specificity of the host recognition response. Four of the five experimental populations exhibited a statistical preference for test solutions from their respective hosts. The specificity of these responses was investigated in a series of experiments in which symbionts from the various host populations were presented with test effluents from organisms closely related to the symbiont's original host or organisms which have been reported as being alternate hosts for *A. pulchra*. Again, worms were exposed to filtered sea water and the test effluents simultaneously.

The results of the experiments for the determination of the specificity of the responses of populations of *Arctonoe* are presented in Table II. The general pattern of the responses is one of rather pronounced specificity which is evident in the responses of the worms living with *Dermasterias* and the two species of *Stichopus*. These worms exhibited a significant response only towards their original host when assayed under these experimental conditions (Tables I and II). In fact, of the nineteen test situations involving worms from five species of hosts, a significant response to an organism other than a worm's original host occurred in only three tests. The first of these involved worms from the sea star *Petalaster* which although they did not respond to their original host (Table I) did respond to another asteroid, *Solaster stimpsoni* (Table II). This is the only incidence of a response by these worms which could in any way be construed as being indicative of "recognition." Finally, worms from the gastropod *Megathura* exhibited two responses which deviated from the general trend of specificity which seems to characterize the host recognition behavior of *A. pulchra*. In one test these worms responded very significantly to an alternate host, the holothurian *Stichopus parvimensis*, in addition to their original gastropod host (Tables I and II). In another test these same worms responded to the gastropod *Haliotis* (Table II); however, since only a few

TABLE I

The responses of Arctonoe pulchra to effluents from its respective hosts

Original host	Distribution			χ^2 Host vs Blank	P	Total # exps.
	Host	Blank	Negative			
Asteroidea:						
<i>Petalaster foliolata</i>	16	16	13	0.0	1	4
<i>Dermasterias imbricata</i>	63	5	21	49.4	<0.005	7
Holothuroidea:						
<i>Stichopus californicus</i>	26	2	9	20.6	<0.005	4
<i>Stichopus parvimensis</i>	193	29	51	120.6	<0.005	16
Gastropoda:						
<i>Megathura crenulata</i>	91	15	43	54.4	<0.005	10

TABLE II
The response of Arctonoe to non-host effluents

Worms from	Test organisms	Distribution			χ^2 Host vs Blank	P	Total # exps.
		Host	Blank	Negative			
<i>Petalaster foliolata</i>	Holothuroidea						
	<i>Stichopus californicus</i>	14	11	12	0.36	0.5-0.75	3
	Asteroidea						
	<i>Dermasterias imbricata</i>	7	10	21	0.52	0.25-0.5	2
	<i>Pteraster tessellatus</i>	11	10	21	0.05	0.75-0.9	4
	<i>Solaster stimpsoni</i>	20	9	25	4.16	<0.05	4
<i>Dermasterias imbricata</i>	Holothuroidea						
	<i>Stichopus parvimensis</i>	42	30	75	2.0	0.25-0.5	8
	Asteroidea						
	<i>Patiria miniata</i>	1	1	24	0.0	1	2
	Gastropoda						
	<i>Megathura crenulata</i>	3	4	23	0.14	0.5-0.75	4
<i>Stichopus californicus</i>	Holothuroidea						
	<i>Cucumaria miniata</i>	12	15	22	0.33	0.5-0.75	2
	Asteroidea						
	<i>Dermasterias imbricata</i>	3	7	5	1.6	0.1-0.25	2
	<i>Petalaster foliolata</i>	8	8	23	0.0	1	3
	<i>Pteraster tessellatus</i>	13	8	17	1.19	0.25-0.5	4
	<i>Solaster stimpsoni</i>	20	11	34	2.6	0.1-0.25	5
<i>Stichopus parvimensis</i>	Asteroidea						
	<i>Patiria miniata</i>	4	10	16	2.56	0.1-0.25	2
	<i>Dermasterias imbricata</i>	64	60	33	0.13	0.5-0.75	6
	Gastropoda						
	<i>Megathura crenulata</i>	15	32	23	6.8	0.01-0.05	4
<i>Megathura crenulata</i>	Holothuroidea						
	<i>Stichopus parvimensis</i>	50	9	23	28.4	<0.005	6
	Asteroidea						
	<i>Dermasterias imbricata</i>	12	9	31	0.44	0.5	5
	<i>Patiria miniata</i>	8	5	17	0.7	0.25-0.5	2
	Gastropoda						
	<i>Kelletia kelletia</i>	1	5	24	2.66	0.1-0.25	1
	<i>Iliotia rufescens</i>	15	4	33	6.36	<0.025	3

of the worms offered this test organism made a choice, these results may not be indicative of attraction to this species by these worms.

Discrimination between alternate hosts. The host recognition responses of *Arctonoe* were quite specific when worms were offered a host *versus* blank choice situation. As a further test of the specificity of these responses, another series of experiments was performed in which specimens of *Arctonoe* were simultaneously exposed to effluents from their original host and an alternate host. The results of these experiments are presented in Table III.

These results confirm the observation of specificity in the host recognition responses of these symbionts. In all cases except one the worms exhibited a statis-

tically significant preference for the arm of the Y containing effluent from their original host. The presence of an alternate host in the system apparently did not affect the behavior of these worms. Furthermore, the results of the experiments in which *Arctonoe* from *Megathura* was presented effluents from *Megathura* and *Stichopus* provide additional evidence that these worms respond positively to this alternate host species; the worms distributed themselves randomly in the Y-maze. It should be noted that in these experiments control experiments employing worms from the respective hosts insured that the various test effluents were attractive to the test organisms' own symbionts.

Although no quantitative evaluations were made, there were no obvious behavioral variations among any of the worms employed in these experiments which could be directly attributed to differences in age (size) or sex among the worms. Worms as small as 10 mm responded similarly to those as large as 50–60 mm. Likewise, no obvious seasonal variations in these host recognition responses occurred.

Since the protocol utilized in these experiments attempted to exclude any form of information exchange other than by chemical means between the test organisms and *Arctonoe*, the data from these investigations indicate that certain of the hosts for this symbiont release some substance(s) which acts as an attractant for these symbiotic polychaetes. No critical evidence is available to ascertain whether qualitative and/or quantitative differences exist among the attractants. One might reasonably expect qualitative differences to occur among the attractants since the host recognition responses are quite specific. Also, this specificity is evident even when test effluents are "brewed" for much shorter time periods than the 20–24 hour interval used in these experiments, a fact which might indicate that quantitative differences alone do not supply the requisite information to effect the observed behavioral specificity of these symbionts. In addition, the phylogenetic diversity

TABLE III

Specificity of the responses of Arctonoe pulchra to effluents from alternate hosts

Worms from:	Distribution when offered host vs alternate host		χ^2	<i>P</i>	Total # Exps.
<i>Dermasterias imbricata</i>	Dermasterias 57	Megathura 3	46.6	<0.005	5
	Dermasterias 225	Stichopus 25	160	<0.005	19
<i>Stichopus parvimensis</i>	Stichopus 52	Megathura 8	32.2	<0.005	5
	Stichopus 226	Dermasterias 27	190	<0.005	12
<i>Megathura crenulata</i>	Megathura 19	Dermasterias 4	9.7	<0.005	2
	Megathura 22	Stichopus 18	0.4	0.5–0.74	3

of the hosts involved in these associations might result in there being greater molecular diversity among the attractants.

Effects of previous experience

Specificity in the host recognition responses of *Arctonoe pulchra* indicates that this worm is capable of discriminating its respective host from among an array of alternate host or non-host species. This discrimination implies that these worms possess sensory and/or integrative apparatus which permits this discrimination. Regardless of which parameter of the chemical signal from a particular host organism provides the requisite information to effect this discrimination, one wonders whether this behavior is genetically fixed or whether the previous experience of individual worms could modify their responses. Thus, the question of whether some conditioning phenomenon might affect this host recognition behavior was investigated. In the experiments which follow the assay for an effect of various experimental parameters on the specificity of host recognition consisted of exposing worms from a particular host to effluents from two species of host (their original host and one alternate host) simultaneously in the Y-maze. In all cases the appropriate control experiments were conducted to insure that the test effluents were attractive; otherwise, the experimental procedure followed that outlined in Methods.

Effect of long-term physical contact with an alternate host. Field collection data (Dimock, 1970) indicate that associations between *Arctonoe* and its hosts start early in the life of the symbionts. Could such long-term intimacy with a host have any effect on the host recognition behavior exhibited by adult worms?

Previous experience with *Arctonoe pulchra* had clearly indicated to us that the response of this worm to at least two species of hosts was not significantly altered either by maintaining the worms for long periods in the laboratory under ordinary holding conditions, *i.e.*, on their respective hosts in running sea water, or by keeping the worms completely isolated from any physical or chemical contact with any host. In fact, worms which had been isolated from their host continued to respond significantly and specifically to that host after as much as five weeks' isolation. Thus, we performed a series of experiments to determine if intimate physical contact might modify a worm's response to a particular species of host.

A series of reciprocal experiments involving worms from the sea cucumber *Stichopus parvimensis* and the sea star *Dermasterias imbricata* was performed. Worms from the sea cucumber and the sea star were removed from their host, tested in the choice apparatus for their initial host preference and then placed upon the respective alternate host which previously had been freed of all worms. Thus, worms which had previously been on *Stichopus* were placed on *Dermasterias* and vice versa. These worms were kept on the alternate hosts in the laboratory and their host preference behavior was monitored at intervals over a four-week period. The results of these experiments are presented in Table IV.

The data indicate that, indeed, intimate contact between these worms and an alternate host does affect the subsequent behavior of *Arctonoe*. The results of experiments 1 through 3 in Table IV clearly show that by the end of the experiment, either 3 or 4 weeks, the worms no longer exhibited a significant preference for their original host. In all experiments the worms distributed themselves ran-

TABLE IV
Conditioning worms from one host to an alternate host

Worms initially from:	Exp. #	Time on alternate host	Distribution			Host χ^2 / Host	P
			<i>Dermasterias</i>	<i>Stichopus</i>	Negative		
<i>Stichopus parvimensis</i>	1	0 weeks	2	29	10	23.4	<0.005
		3 weeks	8	9	1	0.06	0.75-0.9
	2	0 weeks	4	26	0	10.8	<0.005
		2 weeks	6	18	6	6.0	0.01-0.025
		3 weeks	7	19	2	5.54	0.01-0.025
		4 weeks	10	9	7	0.05	0.75-0.9
	3	0 weeks	2	27	3	21.4	<0.005
		2 weeks	5	20	1	8.74	<0.005
3 weeks		6	13	5	2.56	0.1-0.25	
4	0 weeks	5	28	1	10.9	<0.005	
	2 weeks	21	8	3	5.82	0.01-0.025	
<i>Dermasterias imbricata</i>	5	0 weeks	29	3	5	21.1	<0.005
		3 weeks	17	9	5	2.46	0.1-0.25
		4 weeks	6	17	5	5.26	0.01-0.025
	6	0 weeks	22	3	5	14.4	<0.005
		2 weeks	7	15	7	2.9	0.05-0.1
		3 weeks	5	18	1	7.32	<0.01
		4 weeks	4	10	1	2.56	0.1-0.25

domly in the maze. It should be noted, however, that control experiments indicated that the test effluent from each host organism was attractive to "naive," *i.e.*, unconditioned, worms from the respective hosts.

The results of the 4th experiment in this series suggested that this conditioning process could have an even more pronounced effect on *Arctonoe* from *Stichopus*. In this experiment (Table IV, Exp. 4) not only did the worms lose their preference for their original host, but they actually switched and exhibited a statistically significant attraction towards the alternate host *Dermasterias*. These data clearly indicate that the worms somehow had become "conditioned" to the new host and thus were attracted to it.

The reciprocal experiments which involved placing worms from *Dermasterias* on *Stichopus* yielded similar results (Table IV, Exps. 5 and 6). Once again the effect was dramatic. In both of these experiments the worms underwent a profound change in their host preference; not only did fewer forms choose their original host, but a significant number of the worms chose the "conditioned" host in preference to their original.

Effect of long-term olfactory exposure on host preference. The observations from the foregoing experiments would appear to indicate that intimate contact between *Arctonoe* and a host species influences the subsequent responses of that worm to host effluent. It thus seemed of interest to determine if actual physical

TABLE V

Effect on Arctonoe from Stichopus of long-term olfactory exposure to Dermasterias

		Choice—1			P Choice	Choice—2			P Choice
		<i>Sti- chopus</i>	<i>Derma- sterias</i>	Nega- tive		<i>Derma- sterias</i>	Blank	Nega- tive	
Experimental worms	Initial	28	1	1	<0.005	14	13	3	0.75-0.9
	2 weeks	26	2	2	<0.005	11	19	0	0.1 -0.25
	3 weeks	20	8	1	<0.05	11	16	2	0.75-0.9
Control worms	Initial	26	2	2	<0.005	12	14	4	0.75-0.9
	2 weeks	24	2	2	<0.005	15	10	3	0.5 -0.75
	3 weeks	22	5	1	<0.005	12	11	5	0.75-0.9

contact between host and symbiont was necessary to effect a change in worm behavior, or whether this change might be brought about simply by exposing the worms to "host odor" for an extended period. In addition to the biological interest of such a question, a quite practical reason for such a determination exists. If *Arctonoe* is influenced by what it smells, might not its responses to later olfactometer tests be affected by exposure during an earlier test to concentrated test effluent?

Worms from *Stichopus parvimensis* were maintained in individual plastic tubes upon a two-layered platform of fiberglass screen suspended in a 15 gallon aquarium of aerated running sea water. Twelve sea stars, *Dermasterias imbricata*, were held in the lower half of the aquarium by the screened barrier. The two layers of screen were separated 1½ inches by a wooden frame. Thus, the worms in the tubes in the upper portion of the aquarium were exposed to rather concentrated *Dermasterias* "odor" but were not permitted physical contact with these stars. At various times during this experiment the sea water stopped running into the aquarium, at these times the worms probably were exposed to an even higher concentration of this alternate host's odor. A control group of *Arctonoe* was held under similar conditions except that no sea stars were placed with them in the aquarium. The experiment was run for three weeks.

The effects of these experimental conditions on the subsequent behavior of *Arctonoe* were monitored in two ways. The first assay was identical to that used in the previous experiments, *i.e.*, worms were exposed to effluents from their original host, *Stichopus*, and the alternate host, *Dermasterias*, simultaneously. The second assay was similar except that effluent from *Dermasterias* was offered to the worms simultaneously with filtered sea water. The work of Thorpe and Jones (1937) suggests that although test organisms might maintain a preference for their original host when offered the original and the "conditioned" host simultaneously, presentation of the "conditioned" host and a "blank" in the olfactometer might detect some effect of the exposure to the alternate host. The results of these analyses are summarized in Table V.

The response of the experimental group of worms to the *Stichopus-Dermasterias* choice situation following three weeks exposure to *Dermasterias* effluent differed significantly from the initial response of this group ($\chi^2 = 4.99$, $P < 0.05$ by a 2×2 contingency table with Yates' correction factor). However, the response

of the experimental group at three weeks did not differ significantly from that of the control worms at three weeks ($\chi^2 = 0.002$ by the same test). Both groups of worms exhibited a significant preference for *Stichopus*. In addition, an analysis of the data from the *Dermasterias*-blank choice situation yielded no discernible effect of the continuous olfactory exposure on the behavior of the worms, in spite of the fact that this test is perhaps more sensitive than the two-host choice situation. Thus, the results indicate that the long-term olfactory exposure had no effect on *Arctonoe*. Therefore, it is unlikely that exposure of *Arctonoe* to concentrated effluents in one test affects the subsequent behavior of this worm.

DISCUSSION

Regardless of where in the stimulus-response chain the integration of information necessary to effect the observed behavioral specificity of *A. pulchra* occurs, the data from this study clearly indicate that, indeed, several populations of this symbiont differ in their host recognition behavior from that of conspecifics. Of the five populations of this symbiotic polychaete investigated, the group living with the mud-star *Petalaster foliolata* was singular in its lack of overt host recognition. These observations agree with earlier results obtained by Davenport (1950) for worms from this sea star. However, in this study, unlike Davenport's, only intact, apparently healthy mud-stars were used. Thus, although Davenport's suggestion that injured stars may release some "injury substances" which inhibit the worm's response may be true, such a mechanism cannot be invoked to explain the lack of response of these worms observed in this study. It is possible that associations between this star and *Arctonoe* do not occur as the result of some active host recognition behavioral mechanism. These mud-stars appear to provide the predominant solid substrate in their soft mud habitat, and, if larval or juvenile worms were first attracted to this total environment (Laing, 1937), associations with this host star might come about as a result of random encounters between the worms and this "substrate."

The other experimental populations of *Arctonoe* exhibited statistical preferences in the Y-maze which clearly indicated host recognition. These host recognition responses furthermore seem to be quite specific. Worms from the sea star *Dermasterias* and the two species of *Stichopus* failed to respond to test organisms closely related to their original hosts or to species which function as alternate hosts for this symbiont, hosts which in turn are attractive to their respective symbiotic partners. The observed specificity of this host recognition was maintained even when symbionts were offered a choice between alternate hosts simultaneously (Table III).

There were, however, several exceptions to the observed specificities in the responses of these symbionts. One notable exception was the response of worms from the mud-star *Petalaster* towards the asteroid *Solaster stimpsoni* (Table II). As previously indicated this asteroid has been reported as being a host for *A. pulchra*, but the significance of the observed response of these worms is at present not clear.

The other exceptions to this overall trend of pronounced specificity in these host recognition responses occurred within populations of *Arctonoe* living with the gastropod *Megathura*. The responses of these worms to the gastropod *Haliotis rufescens* (Table II) might suggest that these worms simply were responding to

some generalized molluscan attractant. However, the high proportion of worms not making any choice when exposed to this test organism and the lack of response by these worms to the gastropod *Kelletia*, indicate that this is not the case. Further investigations must be undertaken to determine if in fact this observed response is indicative of an attraction to this mollusc.

The responses of *Megathura* worms to effluents from the alternate host *Stichopus parvimensis* provide the most puzzling results of the experiments utilizing these symbionts. This alternate host species, among all of the test organisms, was the only one which evoked a significant response from a population of *A. pulchra* that also responded to its original host. The response to *Stichopus* was further verified by the behavior of these worms when exposed simultaneously to their original host, *Megathura*, and *Stichopus* (Table III). Under these conditions the worms responded equally to the test organisms, *i.e.*, they distributed themselves randomly in the arms of the Y-maze. The significance of such behavior is at present unknown.

Experiments described above clearly indicate that a population of worms may be conditioned to an alternate host. Inherent in most learning theory is the concept of reinforcement. However, Thorpe and Jones (1937), in a study of host selection in parasitic insects, concluded that simple exposure to a certain chemical environment at some time during an organism's life cycle tended to increase responses to those chemical stimuli in subsequent laboratory tests. The nature and function of this olfactory conditioning in insects were explored in a subsequent series of investigations (Thorpe, 1938, 1939, 1963).

Olfactory conditioning has not previously been reported for annelids. Indeed, among the polychaetes no form of learning other than habituation has been conclusively demonstrated except perhaps maze learning (Evans, 1965, 1966a, 1966b; Wells, 1968). Therefore, the results of the present study are of interest since they show an additional capability for plasticity in the behavioral repertoire of these annelids.

Extensive experimental analyses must be performed before much can be said about the mechanisms involved in effecting this conditioning. The data from the present study suggest that close physical contact may be required to effect this change in olfactory response. Continuous exposure to the effluent from *Dermasterias* had no discernible effect on the behavior of *Arctonoe* (Table V). Wood (1968) found the same thing to be true regarding conditioning of the oyster drill *Urosalpinx*. The lack of effect of this exposure may, however, simply be a function of the time course employed in these experiments. Physical contact with the host may be a more effective means of bringing about conditioning. Monteith (1955) found physical contact more effective for conditioning an insect than olfactory exposure.

Regardless of the mechanisms involved in effecting conditioning this phenomenon is perhaps of fundamental significance to these symbionts. Once an intimate association between a worm and a host is effected, the worm could become conditioned to some chemical signal from the host, a signal which might serve to bind the partners together. If for instance young worms were initially attracted non-specifically to all of the hosts in a particular habitat, they might then develop specificity in their host recognition behavior as a result of this conditioning phenomenon. At present one may only theorize about the adaptive significance of

this capability for modification of the host recognition behavior of these symbionts. Further analysis of the role of conditioning in the life cycle of these organisms will await rearing of the worms and a determination of what parameters effect the onset of host recognition behavior and the specificity in these responses exhibited by this polychaete.

Recent investigations in our laboratory (Dimock, 1970) have indicated a possible role for the host recognition responses of adult *A. pulchra*. The distribution of large worms (>20 mm) on two of the hosts of this symbiont, *Stichopus parvimensis* and *Megathura crenulata*, is very regular at one large worm per host. Furthermore, experimental evidence clearly indicates that these large worms can regulate this density, apparently through intraspecific aggression. Thus, since small worms are present at much higher densities on these hosts than are large, some worms may at some point in time be forced to leave a host organism and colonize another. Certainly, an obligate symbiont must find another host or die if it for any reason becomes separated from its host. Therefore, the possession of some mechanism for effecting host recognition and relocation may be of selective advantage to this symbiont.

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SUMMARY

1. Several populations of the symbiotic polychaete *Arctonoe pulchra* were examined with regard to the ability of these worms to detect chemical signals emanating from their hosts. Four of the five experimental populations exhibited chemotactic responses which can be interpreted as host recognition.

2. The host recognition responses were for the most part very specific in that worms consistently chose their original host when presented effluents from a variety of organisms in a Y-maze choice apparatus.

3. The specificity of these chemotactic responses could be affected by the previous experience of individual worms. That is, worms could be conditioned to respond to an organism which previously was unattractive to them.

4. The mechanisms involved in effecting this modification of behavior are not clear. However, the results of this study indicate a heretofore unknown behavioral capability among these polychaetes.

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